VIRAL VECTOR PRODUCTION SYSTEM

FIELD OF THE INVENTION

[0001] The invention relates to the production of viral vectors. In particular, the invention relates to viral vector cell production systems engineered to express and secrete a nuclease into cell culture media during the vector manufacturing process.

BACKGROUND TO THE INVENTION

[0002] As indicated above, the present invention relates to production cells, the preparation thereof and uses thereof. A production cell is sometimes also referred to as a host cell or host production cell. The production cells are useful in inter alia gene therapy.

[0003] Gene therapy broadly involves the use of genetic material to treat disease. It includes the supplementation of cells with defective genes (e.g. those harbouring mutations) with functional copies of those genes, the inactivation of improperly functioning genes and the introduction of new therapeutic genes.

[0004] Therapeutic genetic material may be incorporated into the target cells of a host using vectors to enable the transfer of nucleic acids. Such vectors can be generally divided into viral and non-viral categories.

[0005] Viruses naturally introduce their genetic material into target cells of a host as part of their replication cycle. Engineered viral vectors harness this ability to enable the delivery of a nucleotide of interest (NOI) to a target cell. To date, a number of viruses have been engineered as vectors for gene therapy. These include retroviruses, adenoviruses (AdV), adeno-associated viruses (AAV), herpes simplex viruses (HSV) and vaccinia viruses.

[0006] In addition to modification to carry a nucleotide of interest, viral vectors are typically further engineered to be replication defective. As such, the recombinant vectors can directly infect a target cell, but are incapable of producing further generations of infective virions. Other types of viral vectors may be conditionally replication competent within cancer cells only, and may additionally encode a toxic transgene or pro-enzyme.

[0007] The use of viral vectors for delivery of therapeutic genes is well known and wide-ranging across indications. In particular, gene therapy advances and products are now an important part of our global healthcare markets. Contemporary gene therapy vectors based on RNA viruses such as γ-Retroviruses and Lentiviruses, and DNA viruses such as Adenovirus and Adeno-associated virus (AAV) have shown promise in a growing number of human disease indications. These include ex vivo modification of patient cells for haematological conditions, and in vivo treatment of ophthalmic, cardiovascular, neurodegenerative diseases and tumor therapy or immunotherapy. Other viral vectors such as viruses based on Poxviruses and Avian viruses are widely used in human and animal vaccinations.

[0008] Retroviral vectors, developed as therapies for various genetic disorders, continue to show increasing promise in clinical trials and now a few form the basis of approved therapeutic products. Currently there are over 459 human clinical trials involving retroviral gene therapy registered in the Journal of Gene Medicine database; 158 gene therapy clinical trials are using lentiviral vectors (http://www.abedia.com/wiley/vectors.php, updated in April, 2017). Strimvelis received marketing authorisation from the European Commission on 26 May 2016; Strimvelis is a product for treatment of ADA-SCID based on patient $CD34^+$ cells transduced ex vivo with retroviral vectors expressing the ADA gene. Kymriah (USAN: tisagenlecleucel) received approval from the FDA on 30 Aug. 2017; Kymriah is a product for the treatment of patients up to 25 years old with refractory ALL. Papers on retroviral gene therapy include Wang X, Naranjo A, Brown C E, Bautista C, Wong C W, Chang W C, Aguilar B, Ostberg J R, Riddell S R, Forman S J, Jensen M C (2012) J Immunother. 35(9):689-701, Hu Y, Wu Z, Luo Y, Shi J, Yu J, Pu C, Liang Z, Wei G, Cui Q, Sun J, Jiang J, Xie J, Tan Y, Ni W, Tu J, Wang J, Jin A, Zhang H, Cai Z, Xiao L, Huang H. (2017) Clin Cancer Res. 23(13):3297-3306, Galy, A. and A. J. Thrasher (2010) *Curr Opin Allergy Clin Immunol* 11(6): 545-550; Porter, D. L., B. L. Levine, M. Kalos, A. Bagg and C. H. June (2011) *N Engl J Med* 365(8): 725-733; Campochiaro, P. A. (2012) *Gene Ther* 19(2): 121-126; Cartier, N., S. Hacein-Bey-Abina, C. C. Bartholomae, P. Bougneres, M. Schmidt, C. V. Kalle, A. Fischer, M. Cavazzana-Calvo and P. Aubourg (2012) *Methods Enzymol* 507: 187-198; Sadelain, M., I. Riviere, X. Wang, F. Boulad, S. Prockop, P. Giardina, A. Maggio, R. Galanello, F. Locatelli and E. Yannaki (2010) *Ann NY Acad Sci* 1202: 52-58; DiGiusto, D. L., A. Krishnan, L. Li, H. Li, S. Li, A. Rao, S. Mi, P. Yam, S. Stinson, M. Kalos, J. Alvarnas, S. F. Lacey, J. K. Yee, M. Li, L. Couture, D. Hsu, S. J. Forman, J. J. Rossi and J. A. Zaia (2010) *Sci Transl Med* 2(36): 36ra43 and Segura M M, M. M., Gaillet B, Gamier A. (2013) *Expert opinion in biological therapy*).

[0009] Important examples of such vectors include the gamma-retrovirus vector system (based on MMLV), the primate lentivirus vector system (based on HIV-1) and the non-primate lentivirus vector system (based on EIAV).

[0010] Reverse genetics has allowed these virus-based vectors to be heavily engineered such that vectors encoding large heterologous sequences (circa 10 kb) can be produced by transfection of mammalian cells with appropriate DNA sequences (reviewed in Bannert, K. (2010) *Caister Academic Press:* 347-370).

[0011] Engineering and use of retroviral vectors at the research stage typically involve the production of reporter-gene vectors encoding, for example, GFP or lacZ. The titres of these clinically irrelevant vectors are usually in the region of 1×10^6 to 1×10^7 transducing units per mL (TU/mL) of crude harvest material.

[0012] The manufacture of viral vectors for human gene therapy and vaccination is well documented over the last several decades in scientific journals. Well known methods of viral vector manufacture include the transfection, such as transient transfection, of primary cells or mammalian/insect cell lines with vector DNA components, followed by a limited incubation period and then harvest of crude vector from culture media and/or cells. Transient transfection requires that the viral genes necessary for the production of viral vectors are introduced into a production cell (for example, HEK-293) via plasmids by transfection. Often, each component required for vector production is encoded by separate plasmids, partly for safety reasons, as it would then require a number of recombination events to occur for a replication competent virus particle to be formed through the production process.

[0013] After transfection, incubation & harvest, viral vector virions are then purified and concentrated from crude